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Effects of Selection for Color Intensity on Antioxidant capacity in Maize (*Zea mays* L.)

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ABSTRACT

Maize kernels with diverse colors have pigments and other substances with antioxidant capacity that can be highly beneficial for human health. Our objectives were to analyze antioxidant capacity of the different maize kernel colors, to investigate if selection for color intensity increases antioxidant capacity and to check the effect of bakery processing on antioxidant capacity. Pigment extracts along with hydrophilic fraction showed the highest antioxidant capacity, whereas the lipophilic fraction does not significantly contribute to antioxidant capacity. Visual selection for kernel color increased anthocyanin content from 0.55 to 1.13 μmol/g of fresh weight in one unique cycle of selection. The antioxidant capacity observed in bread was halved of that observed in the raw flour. However, during the bakery process the flour is mixed with other ingredients which could cause the dilution of the antioxidant capacity. Therefore, our results suggest that the traditional method for making maize bread out of whole maize flour have not significant effects on antioxidant capacity.

Key words: Anthocyanins, antioxidant capacity, bread, carotenoids and maize.

INTRODUCTION

Maize (*Zea mays* L.) is used as food in many countries. In some of them such in Spain or Portugal bread is made from whole maize flour. Although most consumers prefer white maize, other colors are also used for making bread (Revilla et al. 2012; Revilla et al. 2008). Colored maize has different pigments and other substances with antioxidant capacity that can be highly beneficial for human health. There is a clear relationship between pigment content and antioxidant capacity and several authors have reported that a higher content of pigment compounds in maize kernels is associated to higher antioxidant capacity (Lopez-Martinez et al. 2009; Hu and Xu 2011; Zilic et al. 2012).

Many food products are made from maize, such as breakfast cereals, snacks, flour or diverse bakery items (Revilla et al. 2008). Maize is mainly a basic source of carbohydrates and also is a convenient source of minerals, vitamins and antioxidant compounds that can be worthwhile to exploit. Maize is rich in oil, similar to oat and millet; it also has a high content of potassium, sodium, chlorine and sulphur. Furthermore, maize protein is poor in lysine and tryptophan but rich in methionine and cysteine (Enwere 1998).

Žilić et al. (2012) found considerable differences in phytochemical contents and antioxidant capacity between a set of maize genotypes. Hu and Xu (2011), Kuhnen et al. (2011), and Xu et al. (2010) also found that the distribution of carotenoids or anthocyanins significantly differed among classes of colors. Pigments and other antioxidant compounds are genetically regulated and can, therefore, be improved through selection. Maize pigments have been reported to have beneficial properties for health due to their high antioxidant capacity which is related to prevention of mutagenesis or cancer and antiradical activities (Adom and Liu 2002). These pigments are important in functional foods as agents to prevent diabetes and obesity (Tsuda et al. 2003).

Pigment content and antioxidant capacity can be altered by maize processing during food manufacturing. Indeed, several authors have showed that the effect of nixtamalization on antioxidant compounds is a reduction of both content and activity of antioxidant products (Del Pozo-Insfran et al. 2006; de la Parra et al. 2007; Lopez-Martinez et al. 2011). Nevertheless, nixtamalization implies removing some parts of the kernel, while maize bread is made with whole flour. Furthermore, Lopez-Martinez et al. (2012) concluded that antioxidant capacity was affected significantly by nixtamalization, but not by processing dough into tortilla and tortilla chips. Besides nixtamalization, other processes can alter total anthocyanin content, as anthocyanins are sensitive to degradation by high pH, light and temperature (Li et al. 2011). Particularly, pigment destruction is logarithmic on time while heating at a constant temperature. Therefore a short time and high temperature are recommended to retain maximum total anthocyanin content in most studies (Francis 1989).

Maize color can be easily identified visually and, therefore, can be increased through phenotypic selection. Selection for color intensity should efficiently increase the amount of pigments and alter the composition of the diverse intermediates involved in pigment metabolism. The effects of such selection on antioxidant capacity would thus depend on the overall variation of the balance of antioxidant substances. As far as we know, there are no reports of visual selection for increasing color or demonstrating if such selection has direct effects on antioxidant capacity. Furthermore, no previous report has shown if the antioxidant capacity remains after bakery processing. Therefore, our objectives were to analyze antioxidant capacity of the different maize kernel colors, to investigate if selection for color intensity increases antioxidant capacity and to check the effect of bakery processing on antioxidant capacity.

MATERIALS AND METHODS

Plant material

Antioxidant capacity of the different maize kernel colors was tested on random samples of 100 kernels with five different colors: white, yellow, pink, purple and black, that could be visually separated. Sample was grinded in an analytical grinding mill (Model A10, IKA, Germany). Two subsamples were taken from each sample and pigment quantification and antioxidant capacity analyses carried out per duplicate. The base population was the multicolor maize population EPS4 that was made by random mating of three local populations (Salcedo, Taboadelo and Cambados) from northwestern Spain with diverse kernel colors. However, given that the population EPS4 has many segregating colors, no kernels with pure white or yellow colors were present and these two classes are not as neat as in pure white or yellow varieties.

In order to investigate if selection for color intensity increases antioxidant capacity, the population EPS4 was also used as the base breeding population for a selection program aimed at increasing color intensity. We used full-sib recurrent selection beginning with 600 plants of EPS4 that were randomly crossed in pairs of plants for obtaining at least 100 two-plant crosses. After harvest, 60 ears were selected for dark color intensity and color uniformity. This procedure was repeated two more times, therefore obtaining three cycles of selection for color intensity.

Finally, we checked the effect of bakery processing on antioxidant capacity by making maize bread from three local populations previously tested as appropriate for bakery and with pure colors (Revilla et al. 2008). These populations were Rebordanes with white kernels, Sarreaus with yellow kernels, and Meiro with black kernels. Bread was made with 600 g of whole grain maize flour, 300 g of whole grain wheat flour, 3 g of lyophilized yeast (*Saccharomyces cerevisae*), 560 g of water, and 19 g of sodium chloride. Maize and wheat flours were mixed with the yeast. The salt was solved in warm water. The water was added to the flour mixture and mechanically kneaded for ten minutes. Fermentation was allowed in the dark, for one hour, covered with a humid cloth. Finally, cooking was made in an electric oven for one hour at 200 °C.

Maize bread was lyophilized and milled. Besides, a random sample of each genotype was also milled and lyophilized for further determination of pigments content and antioxidant capacity. The antioxidant capacity was compared with that of the original maize flour used for making these three breads.

Antioxidant capacity

Hidrophilic extracts were obtained by mixing 10 mg of flour of each sample in 1 ml 80% methanol overnight at 4 °C. Samples were centrifuged at 4000 g and supernatant were recovered. For lipophilic extracts 10 mg of each sample were solved in 1 ml of hexane overnight at 4 °C. After that, samples were centrifuged at 4000 g, supernatant was recovered and hexane was left to evaporate. Pellets were resolved in 1 ml acetone. Antioxidant capacity was also measured in carotenoid and anthocyanin extracts obtained as is explained in the next section. Antioxidant capacity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie &Strain (1996). The working FRAP reagent was prepared by mixing 10 vol of 300 mM acetate buffer, pH 3.6, 1 vol of 10 mM TPTZ in 40 mM hydrochloric acid and 1 vol of 20 mM ferric chloride and then heated at 37 °C. 50 µl of extract were added to 250 µl of freshly prepared FRAP reagent and mixed thoroughly. Readings were taken at 593 nm after 30 min in a Spectra MR™ Microplate Spectrophotometer. Two replications were analyzed for each concentration. After extracting the value of the blank to each sample, absorbance was plotted against concentration. A standard curve was prepared with different concentration of Trolox® (0, 0.008, 0.016, 0.024, 0.032, 0.04 mM). Antioxidant capacity of each sample was normalized to Trolox® equivalents per gram of fresh weight.

Pigment content

Carotenoids were extracted from 100 mg of whole grain maize flour with 1 ml of cold acetone/TRIS buffer solution (80:20 v/v, pH=7.8) overnight at 4 °C. The same procedure was employed for anthocyanin extraction except that cold acidified methanol (1% HCL, v/v) was used instead of acetone/TRIS. After that, samples were centrifuged at 4000 g at 4 °C for 10 min. Both, carotenoid and anthocyanin pigments were quantified from the supernatant spectrophotometrically using a Spectra MR™ Microplate Spectrophotometer. Quantification was performed as described in Sims and Gamon (2002).

Statistical analyses

An analysis of variance for pigments and antioxidant capacity was carried out using the procedure GLM of SAS (SAS Institute Inc. 2008) with grain colors and replication as the classification variables. Comparisons of means were made by using the Fishers' protected LSD at P=0.05. Pearson correlations were calculated between pigments and antioxidant capacity from the five grain color classes with the procedure CORR of SAS. Effects of selection for color intensity on pigment content and antioxidant capacity in the maize population EPS4 were evaluated by using the procedure REG of SAS, being number of cycle the independent variable. A second analysis of variance was carried out for studying the effects of bakery on pigment content and antioxidant capacity in Tuy, Sarreaus, and Meiro, and means were compared with Fisher's protected LSD at P=0.05.

RESULTS AND DISCUSSION

Antioxidant capacity of different maize kernel colors

Maize kernels of different colors differed for pigment content (Table 1). Black kernels had more anthocyanins than any other class of color, followed by purple and pink kernels, while differences between yellow and white kernels were not significant; however, the antioxidant capacity of the anthocyanin extract was neither significantly different between black and purple kernels, nor among pink, yellow and white kernels. Differences among classes were less clear for carotenoids; actually black and purple kernels had similar amounts of carotenoids and their antioxidant capacity was also alike; similarly, the carotenoid content of pink, yellow and white kernels were not significantly different and only the antioxidant capacity of the carotenoid extract from white kernels was significantly lower than that of pink kernels. The hydrophilic fraction, enriched in other metabolites different from anthocyanins or carotenoids, behaved different than both pigment extracts; indeed, the highest antioxidant capacity corresponded to purple kernels, followed by black kernels, with differences among the other three classes being not significant. Finally, the lipophilic fraction had antioxidant activities that were not significantly different among kernel colors.

As we used one single multicolor population with large variability for kernel color, the five classes of kernels colors for pigment content were not clearly separated and there were some intermediate kernels; this was particularly important for the white and yellow kernels. Several authors working with different varieties having different kernel colors found that the distribution of carotenoids or anthocyanins clearly differed among classes of colors (Hu and Xu 2011; Xu et al. 2010; Kuhnen et al. 2011; Zilic et al. 2012). For example, Žilić et al. (2012) found that orange maize had high carotenoid content, while kernels with other colors had low amounts. Furthermore, these authors suggested that some pigments could contribute to phenotypic variation in combination with other pigments, e.g. anthocyanin can increase the intensity of the yellow kernels with carotenoids. Even the lightest colors could contain complex combinations of pigments in small amounts. The relationship between pigment content and antioxidant capacity is not always linear, but normally, darker colors have more antioxidant capacity. For example, Lopez-Martinez et al. (2012) reported that a yellow variety and its corresponding products showed the greatest antioxidant capacity and a purple variety ranked highest among the pigmented varieties for antioxidant content.

Previous reports show that there is a relationship between color and antioxidant capacity (Lopez-Martinez et al. 2009; Hu and Xu 2011; Zilic et al. 2012). Accordingly, correlations between colorant content and antioxidant capacity were generally high. There was a high correlation between antioxidant capacity of anthocyanin and carotenoid extracts (Table 2). Other six correlations were around or above 0.9, including all the correlations between both carotenoid or anthocyanin contents and their antioxidant capacities. The lowest correlations corresponded to the antioxidant capacity of the lipophilic fraction and any other variable. Therefore, the antioxidant capacity of the lipophilic fraction is mostly independent of the pigment composition of the kernel.

Although these correlations between pigment content and antioxidant capacity were higher than those reported by Žilić et al. (2012), these authors also found that a higher content of pigments in the maize kernel contributes to their higher antioxidant capacity. Other authors have also found that dark color is associated to higher antioxidant capacity (Lopez-Martinez et al. 2009; Hu and Xu 2011). Moreover, differences among different dark colors could be due to the specific composition of anthocyanin derivatives (Stintzing et al. 2002).

Effects of selection for color intensity on antioxidant capacity

Visual selection for pigment intensity was effective for increasing anthocyanin content in the first cycle, but not at subsequent cycles (Table 3). However, we cannot discard that further increases on pigment concentration could be achieve by using pigment quantification (with analytical methods) as selection criteria. Accordingly, Burt et al. (2011) concluded that visual selection for carotenoid content was effective for increasing carotenoid content. Although there are no other reports on selection of anthocyanin content in maize grain, this result agrees with Landi et al (2008) who made a selection program for cob color. They also found that just one cycle of selection for cob color led to the fixation of the selected alleles. Landi et al (2008) carried out a divergent selection program for cob color in maize and concluded that it was effective for several traits due to putative QTL linked to one single gene (P1), and that selection is also effective in different genetic backgrounds. The assumption of important additive effects of the genes involved is consistent with the significant linear change resulting from the full-sib recurrent selection for grain color

(Frascaroli and Landi 1998). However, the regressions of anthocyanin content or antioxidant capacity of anthocyanin extracts over cycles of selection were not significant. Neither were significant the correlated responses of carotenoid content or the antioxidant capacity of the carotenoid extract or of the hydrophilic fraction (Table 3). Nevertheless, there is a increasing, though not significant, trend on antioxidant capacity along selection, particularly for the anthocyanin extract and the hydrophilic fraction.

Effect of bakery processing on antioxidant capacity

As bread was made by mixing maize flour with other ingredients, we expected at least a reduction in pigments' content and their respective antioxidant capacity due to dilution. Actually, antioxidant capacity was halved for anthocyanin extracts and for carotenoid extracts in all varieties (Table 4). The antioxidant capacity of the hydrophilic fraction did not follow the same pattern than that of pigments.

On the other hand, the behavior of pigment content was only consistent to expectation for anthocyanin content in the black variety Meiro. Anthocyanin content for the other two varieties and carotenoid content for all varieties was not significantly different between flour and bread and differences seem to be random.

Several authors have shown that nixtamalization reduces pigment content and antioxidant capacity (Del Pozo-Insfran et al. 2006; de la Parra et al. 2007; Lopez-Martinez et al. 2011). Also, temperature and acidity have been reported as agent of antioxidant reduction; indeed, Li et al. (2011) concluded that cookies made out of blue corn and baked with citric acid in the convection oven retained the maximum total anthocyanin content, and by baking rapidly at lower temperatures and adding acidulents, it may be possible to increase residual natural source antioxidants in baked foods. Contrarily, our results suggest that the traditional bakery process tested in this study has no significant effects on pigment or antioxidant capacity.

CONCLUSION

Differences in pigment content are directly related to antioxidant capacity in maize kernels. Such differences in antioxidant capacity are directly due to the pigments and also to the hydrophilic fraction, while the lipophilic fraction was not related to antioxidant capacity of colored kernels. Visual selection for kernel color increases pigment content in one unique cycle of selection but the increase in antioxidant capacity was not significant. Finally, the traditional method for making maize bread out of whole maize flour has not apparent effects on antioxidant capacity beside those due to dilution of flour in the dough.

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Table 1. Comparisons of means from the analyses of variance of five grain colors for pigments and antioxidant capacity in a maize population						
	Pigment content (μmol/g FW)		Antioxidant capacity (μm Trolox/g FW)			
Grain color	Anthocyanins	Carotenoids	Anthocyanin extract	Carotenoid extract	Hydrophilic fraction	Lipophilic fraction
Black	5.375a	1.760a	10.819a	10.956a	10.961b	0.172a
Purple	3.413b	1.677a	11.021a	11.236a	12.921a	0.170a
Pink	0.401c	0.664b	8.268b	9.716b	9.575c	0.145a
Yellow	0.254d	0.914b	7.741b	9.174bc	8.934c	0.177a
White	0.280d	0.750b	7.561b	8.736c	9.575c	0.164a
LSD (0.05)	0.091	0.327	1.259	0.860	1.102	0.043
Means followed by the same letter, within each column, are not significantly different						

Table 2. Pearson correlations between pigments and antioxidant capacity from five grain colors in a maize population

Pigment cont.		Pigment content (µmol/g FW)		Antioxidant capacity (µm Trolox/g FW)		
		Anthocyanins	Carotenoids	Anthocyanin extract	Carotenoid extract	Hydrophilic fraction
	Carotenoids	0.95*				
Antioxidant capacity	Anthocyanin extract	0.93*	0.95*			
	Carotenoid extract	0.88*	0.90*	0.98**		
	Hyd. fraction	0.73ns	0.83ns	0.92*	0.91*	
	Lip. fraction	0.36ns	0.53ns	0.26ns	0.16ns	0.17ns

ns, *, ** not significant, significant at P=0.05, significant at P=0.01, respectively.

Table 3. Effects of selection for color intensity on pigment content and antioxidant capacity in the maize population EPS4

Cycle of selection	Pigment content		Antioxidant capacity (µm Trolox/g FW)		
	Anthocyanins (µmol/g FW)	Carotenoids (µmol/mg FW)	Anthocyanin extract	Carotenoid extract	Hydrophilic fraction
EPS4C0	0.52±0.11	3.93±4.47	2.351±0.266	7.737±0.044	7.587±0.596
EPS4C1	1.13±0.15	3.82±3.00	2.710±0.786	10.037±1.459	7.741±1.049
EPS4C2	1.06±0.18	3.63±1.96	2.961±0.457	11.970±0.488	8.058±1.128
EPS4C3	1.11±0.10	2.41±0.38	3.449±0.869	11.042±1.941	8.380±0.124

Table 4. Effects of bakery on pigment content and antioxidant capacity in three maize local populations: Tuy, Sarreaus, and Meiro, with yellow, orange and black grain, respectively.

Maize population	Substance	Pigment content		Antioxidant capacity (µm Trolox/g FW)		
		Anthocyanins (µmol/g FW)	Carotenoids (µmol/mg FW)	Anthocyanin extract	Carotenoid extract	Hydrophilic fraction
Tuy	Flour	0.05±0.06	2.36±1.18	2.145±0.292	6.719±0.228	3.417±0.201
	Bread	0.06±0.06	2.71±0.40	1.275±0.241	3.997±0.171	5.084±0.169
Sarreaus	Flour	0.03±0.03	2.40±0.45	3.014±1.193	7.015±0.127	5.368±0.909
	Bread	0.04±0.03	3.68±1.59	1.369±0.323	4.234±0.571	10.092±6.300
Meiro	Flour	0.97±0.04	1.87±0.15	2.176±0.159	9.113±0.748	6.773±0.613
	Bread	0.32±0.27	2.12±0.27	1.086±0.748	4.566±0.013	5.375±1.448